Down-Regulation of IgE and IgG4 Antibodies to Tetanus Toxoid and Diphtheria Toxoid by Covaccination with Cellular *Bordetella pertussis* Vaccine[1]

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Pertussis (P) toxin acts as adjuvant for IgE formation against simultaneously administered Ags in animal models. P vaccination may also have an adjuvant impact on IgE formation against coadministered diphtheria (D) and tetanus (T) Ags in humans. Sera of 103 D-T-P-immunized and 319 D-T-immunized children aged 2 years were analyzed for IgE, IgG4, and IgG to D and T (radioallergosorbent test), total IgE and IgE against common inhalant allergens (CAP radioallergosorbent test fluoroenzyme immunoassay). Fewer D-T-P- than D-T-immunized children had sera positive for T-IgE (12.6 vs 53.6%, $p < 0.001$), T-IgG4 (71.6 vs 89.2%, $p < 0.001$), D-IgE (31.0 vs 70.5%, $p < 0.001$), and D-IgG4 (85.2 vs 93.4%, $p = 0.039$). Suppression of T-IgE was not dependent on the cutoff chosen for a positive test result, but was dependent on the proportion of D-T immunizations given with P. The risk for sensitization to common environmental allergens did not differ (odds ratio 0.953, 95% confidence interval 0.815–1.114). No significant differences between D-T- and D-T-P-immunized children were found with regard to T-IgG or D-IgG. In summary, IgE and IgG4 (but not IgG) serum levels to coadministered D- and T-Ags are suppressed among P-immunized children as compared with nonimmunized children. These results suggest that the presence of a microbial product during Ag exposure can down-regulate an IgE/IgG4 response in humans. ***The Journal of Immunology*****, 2001, 167: 2411–2417.**

A rise in the prevalence of allergic diseases has been observed in recent decades in industrialized countries (1). Marked prevalence differences within populations of a close genetic background but profound lifestyle differences suggest that environmental variations are responsible (2, 3). In this context, early childhood vaccinations as planned immunological interventions have attracted interest because they may alter the cytokine milieu and thus render the children more or less susceptible to IgE formation and subsequent IgE-mediated allergy against otherwise harmless environmental Ags (4).

We have shown previously that in German children of a prospectively followed birth cohort (MAS-90), considerable IgE responses are constituents of the regular immune response toward diphtheria (D)[3] and tetanus (T) immunization, however exaggerated in atopics (5, 6). Pertussis (P) toxin and the chemically derived toxoid have a long tradition as an adjuvant for IgE formation against simultaneously administered Ags in animal models (7, 8). Thus, vaccination against P may also have an adjuvant impact on IgE formation against coadministered D and T Ags in humans. Conversely, an inverse association between delayed hypersensitivity to tuberculin and atopy was found among Japanese *Mycobacterium bovis* bacillus Calmette-Guérin (BCG)-vaccinated school children, suggesting that mycobacterial Ags may suppress development of IgE and related diseases (9).

In this study we reanalyzed our data to investigate the effect of heat-killed whole-cell *Bordetella pertussis* vaccine on the humoral immune response to coadministered D and T Ags. At the time of the first immunizations, a large fraction of our cohort parents decided against P-covaccination for their child. This enabled us to compare humoral responses in P-vaccinated and nonvaccinated children. In addition, we studied these responses in BCG-vaccinated and nonvaccinated children.

Patients and Methods

Study population

In 1990, a cohort of 1314 neonates recruited in five German cities (Berlin, Düsseldorf, Freiburg, Mainz, and Munich) was selected for a prospective observational study (MAS-90). Of these, 499 neonates (38%) were selected as being at high risk for atopy (two or more close atopic family members and/or cord blood IgE values above 0.9 kU/L), and the remainder were at random risk.

The cohort infants and their parents were regularly seen for follow-up visits at ages 3, 6, 12, 18, 24, and 36 mo. Parents filled in a questionnaire and gave a structured interview about their children's diseases and atopic symptoms. To keep the reporting bias low, parents kept a diary, recording details of all their children's illnesses. The study coordinators monitored the diary during the regular standardized physical examination by trained study physicians (10).

For this study, cohort children were included in the study if they had received immunizations against D and T before their second birthday, if the records of immunization including the date of each

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[3] Abbreviations used in this paper: D, diphtheria; T, tetanus; P, pertussis; BCG, bacillus Calmette-Guérin; RAST, radioallergosorbent test; RU, RAST units; LF, limit of flocculation units.

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injection were complete, there was no suspected P infection, and the serum volume was sufficient. Because IgG4 analysis was performed at a 1/40 dilution, more material was available for this assay than for the IgE analysis working with undiluted serum. If serum availability was limiting, T assay was given priority. T-IgE was available from all children. T-IgG4 was available from 316/319 DT-vaccinated and from 102/103 DTP-vaccinated children. D-IgE and D-IgG4 were available from 200 and 256 DT-vaccinated and from 58 and 81 DTP-vaccinated children, respectively. For the analyses with respect to BCG vaccination, children with a history of tuberculosis or BCG vaccination after the first DT vaccination were additionally excluded.

Vaccinations

In Germany, P vaccination was not officially recommended before 1991. DT vaccination shots were recommended at 3 and 4 mo of age. A booster shot was recommended 1 year later. For DTP vaccination, immunizations were recommended at ages 3, 4, 5, and 15 mo. However, vaccination history of the children was variable. Corresponding to the cohort character, there was no intervention regarding timing of vaccination and selection of vaccine preparations. Vaccinations were recorded from official vaccination documents that were available for all children of the cohort. An infant with documented vaccination was regarded as vaccinated; all other infants were regarded as nonvaccinated.

D-T vaccinations were performed with vaccines containing D toxoid 30–50 IU, T toxoid 40–50 IU, and aluminum as an adjuvant (aluminum hydroxide or aluminum phosphate, 1.25–1.50 mg). D-T-P vaccinations were performed with a whole cell P vaccine containing D toxoid 50 IU, T toxoid 50 IU, aluminum hydroxide 0.75 mg, aluminum phosphate 0.75 mg, and heat-inactivated *B. pertussis* 4 IU. Vaccination cases included in this study had two or more shots in the first year of life and no P infection.

In Germany, infants believed to be at increased risk of tuberculous infection used to be vaccinated shortly after birth. Infants were vaccinated with an attenuated BCG strain (10^5 bacteria, Copenhagen strain 1331; BCG-Vaccine Behring, Marburg, Germany).

Definition of atopic diseases and allergic sensitization

A basic description of morphological skin phenomena and their localization was used to construct a computer algorithm for the definition of atopic eczema according to the morphological criteria given by Seymour et al. (11). A similar but simpler question sheet was filled out by the mothers regarding the case history of the preceding period (12). Obvious recurrent wheezing bronchitis required at least two wheezing episodes with shortness of breath. Obvious atopic rhinitis was diagnosed in the case of blocked and/or running nose without a cold lasting for two or more months during the preceding observation period, with the diagnosis by a physician (12). A child was considered sensitized if the IgE Ab titer of one or more of the nine allergens tested was $\geq$0.35 kU/L.

Determination of allergen concentration in house dust

The levels of major mite (Der p 1 and Der f 1) and cat (Fel d 1) allergens were determined from domestic carpet dust samples by sandwich ELISA as described previously (13).

Determination of IgE

Venous blood samples were obtained at birth (cord blood), 12, 24, and 36 mo, and serum was separated by centrifugation at 3500 rpm for 13 min. Serum samples were stored at -20°C until analysis. Sera were analyzed for total IgE and specific IgE against nine common allergens (birch t3, grass g6, mite d1, cat e1, dog e2, egg f1, milk f2, wheat f4, and soy f14). Analysis was performed in one laboratory by CAP-radioallergosorbent test (RAST) fluoroenzyme immunoassay (Pharmacia, Freiburg, Germany). The efficiency of CAP test results was tested against skin prick test results for five respiratory allergens (cat, dog, mite, birch, and grass) in a subsample of 418 children at 5 years of age. Skin tests were considered positive if the maximum wheal diameter was >3 mm without reaction of negative control (saline), and the skin index was >0.6 (calculated as the ratio of the diameter of the allergen wheal to the histamine (histamine-dihydrochloride 10 mg/L) wheal). The overall efficiency, calculated as the proportion of concordant positive and negative results, is 92.2%. The sensitivity and specificity are 83.8 and 92.5%, respectively.

D and T IgE and IgG4 Ab assays

D and T toxoid were coupled to cyanogen bromide-activated Sepharose (1 mg protein/g Sepharose; 1 mg corresponds to 1500 limit of flocculation units (Lf) T toxoid and to 1800 Lf D toxoid; Pharmacia Fine Chemicals, Uppsala, Sweden). The Sepharose was suspended at 2 mg/ml in PBS containing 10 mM EDTA, 0.2% Tween 20, and 0.05 mg/ml NaN_3 (IgE), PBS with 0.5% sheep serum (v/v), 4.5% bovine serum (v/v), 0.2% Tween 20 (v/v), 0.3% BSA (w/v), and 10 mM EDTA (IgG4). The RAST for IgE and IgG4 was performed by incubation overnight of 50 ml of serum (IgE undiluted; IgG4 1/40 dilution) with 250 ml of Sepharose, followed by washing with 0.1% PBS-Tween 20 (five times). A second incubation was performed with 500 ml of medium (0.5% sheep serum (v/v); 4.5% bovine serum (v/v); 0.2% Tween 20 (v/v); 0.3% BSA (w/v); 10 mM EDTA in PBS, pH 7.4) plus 50 ml of sheep polyclonal anti-IgE or with 500 ml of medium (0.3% BSA; 0.2% Tween 20; 10 mM EDTA in PBS, pH 7.4) plus 50 ml of monoclonal anti-IgG4 (both radiolabeled with ^{125}I). This was followed by final washing for 12 h with 0.1% PBS-Tween 20 (four times). The percentage of bound ^{125}I was measured with a gamma counter. The results were read from a standard calibration curve for IgE in RAST units (RU) (27, 9, 3, 1, 0.3 RU; 1 RU $\approx$ 1.54 IU/ml). For IgG4 arbitrary RU were calculated by an IgG standard graph from a mixture of samples from two grass pollen-treated patients at dilutions of 1/45, 1/90, 1/180, 1/360, 1/720, and 1/1440. For analysis of immunoglobulins against D and T, serum samples obtained at 2 years of age were analyzed.

Statistical methods

Statistical analysis was performed by using the SPSS for Windows 7.0 (SPSS, Chicago, IL). Pearson's χ^2 tests were applied for the assessment of association in two-dimensional contingency tables (14). Fisher's exact test was used when the expected frequency of any cell was <5. The Mann-Whitney *U* test was used for comparisons of continuous variables. For correlations, Spearman's ρ was calculated. Specific IgE Abs to food and inhalant allergens were grouped into values below detection limit (<0.35 kU/L) and detectable values. Cord blood IgE levels were categorized as not elevated (<0.9 kU/L) or elevated. Total IgE values, if categorized, were grouped into values >75th percentile of estimates for a population-based sample or below (15). Data of IgE and IgG4 Ab measurements are left-skewed and were therefore log-transformed to compute partial correlations. To determine whether subgroup differences in the IgE responses to T and D Ag are related to the time period between the last immunization and IgE Ab measurement, these individually variable periods were grouped into four time intervals. Statistical significance was defined by a two-sided

α level of 0.05. Bonferroni adjustments were used for multiple comparisons.

Results

Study population

We included 422 of the cohort children in our study of which 319 children had received no P vaccination and 103 children whole cell P vaccination. Some of the P-vaccinated group (24 children) had not received P vaccine with all DT shots.

P-vaccinated and nonvaccinated children did not significantly differ with regard to gender, hereditary risk for atopy, cord blood IgE levels, siblings, indoor cat allergen exposure, common childhood infections, or further vaccinations. However, the mite allergen exposure was lower among the P-vaccinated children (Table I).

Children received a median of four shots DT vaccine (range 1–6 shots). The median of DT shots was lower in the DT group than in the DTP-vaccinated group (median, three vs four shots, $p < 0.001$; Table II). One child had a diagnosis of tuberculosis, and four children were BCG-vaccinated after their first DT vaccination. These children were excluded from the calculations in view of a possible effect of BCG on humoral responses to DT. Of the remaining 417 children, 50 (15 DTP-vaccinated) received BCG vaccination before their first DT vaccination (median age at vaccination 29 days old). T-IgE was available from all children. T-IgG4 was available from 316/319 DT-vaccinated and from 102/103 DTP-vaccinated children. D-IgE and D-IgG4 were available from 200 and 256 DT-vaccinated and from 58 and 81 DTP-vaccinated children, respectively.

Table I. *Patient characteristics*

Variable	DT-Vaccinated (n = 319)	DTP-Vaccinated (n = 103)	p Value
Male	51.7	58.3	0.248
Cord blood IgE > 0.9 kU/L (%)	17.8	15.3	0.684
Parents sensitized (%)			
Mother	37.9	32.9	0.420
Father	48.0	43.8	0.613
Family history (%)			
Mother atopic	36.1	34.3	0.750
Father atopic	31.8	32.4	0.911
Sibling atopic	24.2	24.3	1.000
Breastfeeding ≤4 wk (%)	24.1	30.1	0.222
Median cat allergen exposure (Fel d1 μg/g carpet dust, mean 6th/18th month)	69	71	0.246
Median mite allergen exposure (Der p1 + Der f1 μg/g carpet dust, mean 6th/18th month)	253	150	0.024
Siblings at birth (%)			
None	54.0	48.4	
1	35.1	31.5	
≤2	10.9	10.1	0.762
Infections in the first 2 years of life (%)			
Measles	1.6	1.0	1.000
Mumps	2.3	0.7	1.000
Rubella	1.9	1.0	1.000
Further vaccinations in the first 2 years of life (%)			
Measles	66.1	74.8	0.103
Mumps	66.5	75.7	0.078
Rubella	65.8	75.7	0.061
Haemophilus influenzae type B	85.6	82.5	0.452
BCG	7.8	6.8	0.894

Table II. *No. of vaccination shots against T/D among P-vaccinated and nonvaccinated children*

No. Shots	DT (%; n = 319)	DTP (%; n = 103)
1	1.3	
2	16.6	3.9
3	81.5	30.1
4	0.6	63.1
5–6		2.9

P vaccination and IgE response

As compared with the DT-vaccinated group, the mean anti-T serum IgE level was significantly lower in the DTP-vaccinated group (Fig. 1). Accordingly, the proportion of sera with anti-T IgE >0.3 RU/ml was lower in the DTP-vaccinated group (12.6 vs 53.6%, $p < 0.001$). This was not dependent on the cutoff chosen for a RAST-positive result (Fig. 2). Measles-mumps-rubella vaccination was somewhat more prevalent in the DTP-vaccinated group (Table I); however, the group of measles-mumps-rubella-vaccinated children had a similar median anti-T IgE level to that for nonvaccinated children (0.05 vs 0.13 RU/ml; $p = 0.878$).

The correlation between the number of DT shots and anti-T IgE was rather poor ($r_s = -0.025$, $p = 0.614$). Although the number of children incompletely covaccinated with P is not large, the number of DT shots received without P seems to increase the proportion of RAST-positive anti-T IgE levels in a dose-dependent fashion (Fig. 3). This analysis was restricted to children with 3–4 shots, because the numbers of children with 1–2 shots or with 5–6 shots were too small to allow a good comparison between DT and DTP (see Table II).

The time intervals between the last D and T immunization and the blood draw for the D- and T-specific Ab analysis ranged from 1 wk to 20 mo. Among DT-vaccinated children, anti-T IgE serum levels are highest during the first month after immunization and decrease over time to the low level that is found in the sera of DPT-vaccinated children during all time intervals throughout (Fig. 4).

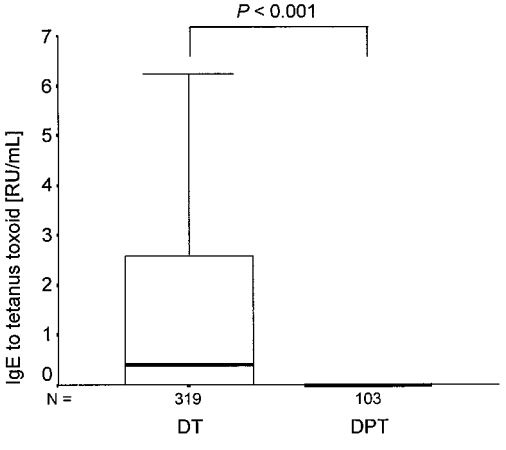


FIGURE 1. Serum IgE Ab level to T toxoid in children aged 2 years vaccinated with DT or DPT. Boxplots represent median, 25th, 75th percentile, and the highest value within the 1.5-fold interquartile interval.

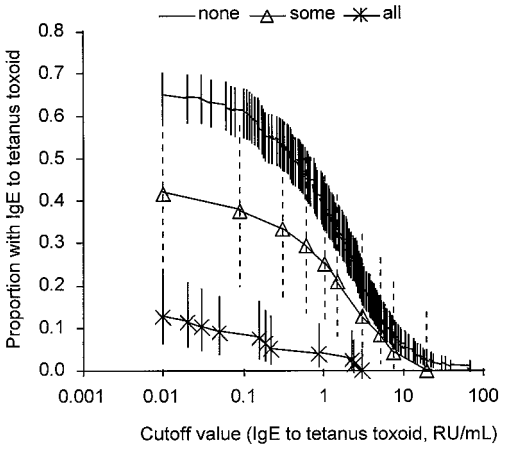


FIGURE 2. Proportion of RAST-positive T-IgE sera among DT- and DTP-vaccinated 2-year-old children in relation to cutoff levels. "all", All DT immunization shots covaccinated with P ($n = 79$); "some", some DT immunization shots covaccinated with P ($n = 24$); "none", none of the DT immunization shots covaccinated with P ($n = 319$). Error bars indicate 95% confidence intervals.

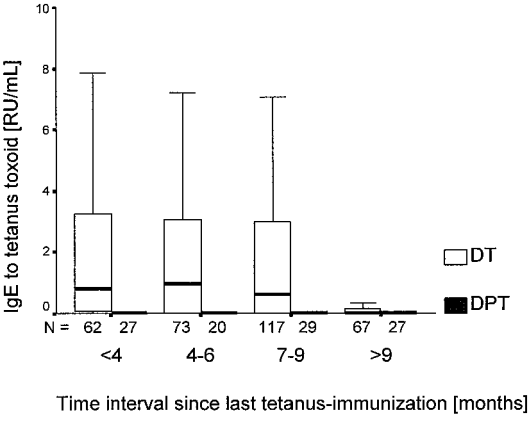


FIGURE 4. Serum IgE Ab level to T toxoid among always DT- or DTP-vaccinated children aged 2 years in relation to the time interval (months) between their last immunization and blood draw. Boxplots represent median, 25th, 75th percentile, and the highest value within the 1.5-fold interquartile interval. ***, $p < 0.001$.

Serum levels of IgE to D are highly correlated with those to T-IgE (Fig. 5). Weaker correlation exists between total serum IgE at 2 years and T-IgE ($r_s = 0.648$; $p < 0.001$) or D-IgE ($r_s = 0.654$; $p < 0.001$) as well as cord blood IgE and T-IgE ($r_s = 0.212$; $p < 0.001$) or D-IgE ($r_s = 0.166$; $p < 0.009$). Like the IgE response to T toxoid, the levels are markedly suppressed in DTP-vaccinated children (Fig. 6).

During the first 2 years of life, total IgE levels are transiently significantly lower in sera of DTP-vaccinated than in sera of DT-vaccinated children. The proportion of children sensitized to common airborne and nutritive allergens is nonsignificantly lower among DTP-vaccinated children at all examination dates (Table III).

P vaccination and IgG4 and IgG response

The rates of seropositivity to IgG4 are higher in both groups than the rates for IgE. As with IgE, the rates of IgG4 to both Ags, D and T toxoid, are statistically significantly suppressed in DPT-vaccinated individuals. However, this effect is less impressive than for IgE (Fig. 7).

The median serum levels of T-IgG4 (P25, P75) are 13.6 RU/ml (2.7 RU/ml, 61.1 RU/ml) among DT-vaccinated children and 2.3 RU/ml (0.0 RU/ml, 6.3 RU/ml) among DTP-vaccinated children ($p > 0.001$). The corresponding levels of IgG4 to D are 22.2 RU/ml (4.0 RU/ml, 82.9 RU/ml) and 5.2 RU/ml (2.1 RU/ml, 17.9 RU/ml; $p < 0.001$) among DT- and DTP-vaccinated children, respectively.

Unlike IgE and IgG4, IgG levels seem not to be influenced by vaccination with P. IgG levels are available from 21 DT-vaccinated and from 14 DTP-vaccinated children. Serum levels of T-IgG (median; P25, P75) do not differ between DT-vaccinated (1.4 RU/ml; 0.3 RU/ml, 2.8 RU/ml) and DTP-vaccinated children (1.1 RU/ml; 0.5 RU/ml, 4.7 RU/ml). The proportion of sera with T-IgG above cutoff is similar in both groups (16/21 DT-vaccinated children and 12/14 DTP-vaccinated children).

BCG vaccination and Ig response

In contrast to P vaccination, serum levels of T-IgE (median; P25, P75) do not statistically significantly differ between BCG-vaccinated (0.4 RU/ml; 0.0 RU/ml, 3.0 RU/ml) and nonvaccinated children (0.1 RU/ml; 0.0 RU/ml, 1.8 RU/ml; $p = 0.451$). Also, T-IgG4

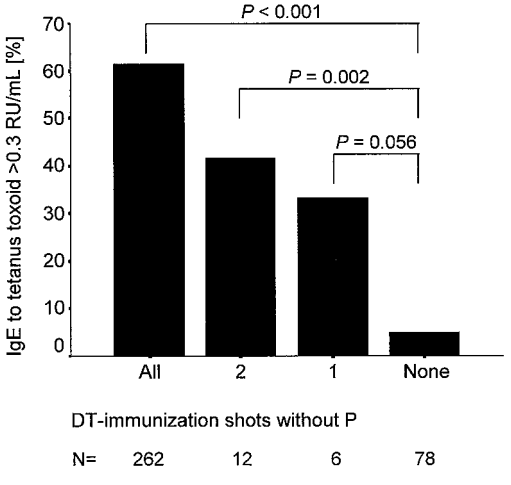


FIGURE 3. Proportion of T-IgE RAST-positive sera among 2-year-old children with three or four DT immunizations in relation to the doses given without P vaccine.

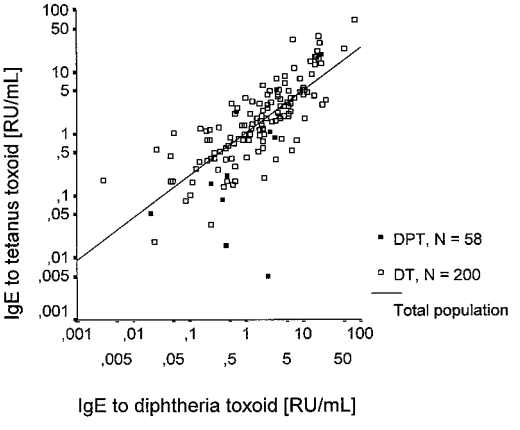


FIGURE 5. Correlation of IgE to T toxoid and to diphtheria toxoid in sera of DT- and DTP-vaccinated children at 2 years of age. The regression line is illustrated for the total population ($r_s = 0.854$; $p < 0.001$).

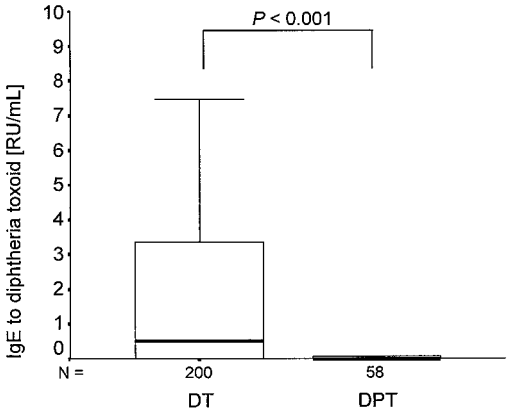


FIGURE 6. Serum IgE Ab level to D toxoid in children aged 2 years vaccinated with DT or DTP. Boxplots represent median, 25th, 75th percentile, and the highest value within the 1.5-fold interquartile interval.

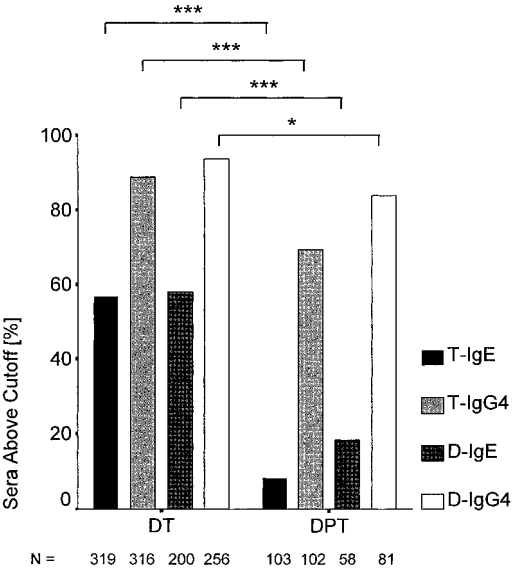


FIGURE 7. Proportion of sera positive for IgE or IgG4 against vaccinated T and D Ags in DT- and DTP-vaccinated children aged 2 years. *, $p < 0.5$; ***, $p < 0.001$.

levels are similar between BCG-vaccinated (13.5 RU/ml; 2.1 RU/ml, 53.2 RU/ml) and nonvaccinated children (8.2 RU/ml; 1.5 RU/ml, 43.6 RU/ml; $p = 0.393$).

However, serum levels of D-IgE (median; P25, P75) are lower in nonvaccinated children (0.2 RU/ml; 0.0 RU/ml, 1.9 RU/ml) than in BCG-vaccinated children (1.6 RU/ml; 0.0 RU/ml, 4.5 RU/ml; $p = 0.024$). D-IgG4 levels are analogous to D-IgE levels and are lower in nonvaccinated children (11.9 RU/ml; 2.7 RU/ml, 50.6 RU/ml) than in BCG-vaccinated children (67.4 RU/ml; 9.5 RU/ml, 170.2 RU/ml; $p < 0.001$).

The baseline characteristics listed in Table I did not differ statistically between BCG-vaccinated and nonvaccinated children. The proportion of P-vaccinated children was equal in both groups. Frequency of respiratory infections during the first three years could not explain this difference (data not shown).

Discussion

Our results demonstrate a striking suppressive effect of whole cell P vaccine on IgE formation to coadministered T and D Ags in early childhood. This finding conflicts with results from animal studies that indicate an IgE-promoting effect of P toxin with regard to coadministered unrelated Ags (8, 16–18). In fact, in humans, not only IgE production (19, 20), but also the promotion of clinical correlates of atopy, has been a concern with P infection (21–23) or P vaccination (24, 25), although a causal relationship has never been established and other studies have failed to show an allergy-promoting effect (26–28).

Transient formation of IgE to the vaccinated Ags is commonly detected and seems to be part of the regular immune response (5, 6, 29–32). The biological function of this has not been elucidated fully but it may be the expression of a Th2-balanced Th1 response that optimizes host defense. Recent data on P have indicated a different response pattern of whole cell and acellular P vaccine. PBMC of whole cell P-immunized children respond with high IFN-γ and low IL-5 secretion, a pattern that has also been found in PBMC of P-infected children (33–35). In contrast, PBMC from children immunized with acellular vaccines show a higher production of IL-4 and IL-5 (34–36). This finding is mirrored in higher levels of IgE to P toxin itself in children immunized with whole cell as compared with those immunized with acellular vaccines (32).

This effect is possibly related to cell wall components such as LPS. A murine study that investigated the influence of LPS exposure on IgE indicated that LPS stimulates B cells to differentiate into IgG-producing plasma cells if LPS is given before the Ig isotype switch has occurred. If given after the switch, LPS directly stimulates those B cells that have been primed to produce IgE (37). Our study indicates that whole cell P vaccine has the potential to suppress the Th2-associated branch of the humoral immune response to Ags if these are administered simultaneously. However, sensitization to common environmental allergens not given with the vaccine seems to be uninfluenced in our study and in other studies (32, 38).

Like IgE, secretion of IgG4 is IL-4-dependent (39). In this study, suppression of IgG4 serum levels in P-vaccinated children parallels suppression of IgE. In contrast, total IgG serum levels do not differ between DT- and DTP-vaccinated children. These

Table III. *Odds ratios (OR) and 95% confidence interval for specific sensitization to common allergens and point prevalences of atopic dermatitis and recurrent wheeze at 12, 24, and 36 mo of life in D/T-vaccinated children with P covaccination (DTP; n = 319) in comparison with children not covaccinated (OR = 1.0; n = 103)*[a]

Age, Condition	OR	95% Confidence Interval	*p* Value
12 mo			
Elevated total IgE	0.424	0.230–0.783	0.008
Sensitization	0.945	0.810–1.124	0.717
Atopic dermatitis	0.973	0.798–1.187	0.966
Recurrent wheeze	0.891	0.728–1.092	0.584
24 mo			
Elevated total IgE	0.418	0.236–0.741	0.004
Sensitization	0.953	0.815–1.114	0.689
Atopic dermatitis	0.875	0.755–1.014	0.225
Recurrent wheeze	0.956	0.773–1.183	0.889
36 mo			
Elevated total IgE	0.688	0.364–1.298	0.314
Sensitization	0.919	0.811–1.040	0.283
Atopic dermatitis	0.850	0.758–0.954	0.037
Recurrent wheeze	0.880	0.753–1.028	0.274

[a] Specific IgE $\leq$ 0.35 kU/L against egg, milk, soy, wheat, birch, timothy, mite, cat (12, 24, 36 mo), or dog (36 mo).

findings support the view that P suppresses the Th2-associated branch of the immune response.

Unlike P, antecedent BCG did not suppress IgE or IgG4 levels. In murine studies, BCG acted as a strong Th1 promotor and suppressed IgE formation to unrelated Ags (40, 41). However, in children, a convincing effect of early BCG vaccination on later development of IgE to common environmental allergens has not been found (42, 43). This may be a dose problem because studies investigating tuberculosis-infected patients suggest a possible effect (44, 45). One study investigating BCG-vaccinated Japanese children reporting an inverse correlation of IgE and skin test reactivity to mycobacterial Ag presumably involved children that were infected with environmental mycobacteria (9). This inverse correlation has not been reproduced in our prospective cohort, or in a study of Norwegian adults (43, 46).

P-vaccinated and nonvaccinated subpopulations were carefully studied for possible confounding and were found to be comparable with regard to common known confounders such as heredity for atopy, allergen exposure, infections, and further vaccinations. There has been a controversial discussion of the extent to which the production of IgE against vaccinated Ags is due to the toxoids themselves or is an effect of alum as adjuvant. However, IgE against T and D toxoids is also detectable after booster doses of nonadsorbed vaccines (47, 48). We could not adjust for different brands of DT vaccine, but there was little variation in the composition of the products available at that time.

The vaccination schedule in 1990 recommended two shots and one booster shot for DT vaccination. For DTP vaccination, three DTP shots and one booster shot were recommended. In our cohort, ~80% of the DT-vaccinated children received three shots and ~70% of the DTP children received four shots. However, the correlation of vaccination doses and T-IgE was rather low. In contrast, we found a dose response-like relationship between the proportion of DT shots with P and T-IgE serum level. This suggests that P vaccine is required at the time when D and T Ags are administered to suppress IgE formation against these Ags.

In summary, we found a strong suppressive effect of P vaccine on IgE and IgG4 formation against coadministered D and T Ags. This effect seems to be dose-dependent. In contrast, BCG vaccination given before DT immunization has no suppressive effect. It may be speculated that administration of common environmental allergens together with a Th1-deviating vaccine results in the prevention or even reversion of IgE formation against these allergens. Although this approach is currently not available as an Ag-specific therapeutic option for children, it is important to note that routine DT vaccination with P does not seem to increase the risk of IgE formation or related disease. Access to these life-saving vaccines should be regarded as a basic human right of every child—atopic or not.

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